3188-Plat

Voltage and Calcium Coupling in the Genesis of Cardiac Afterdepolarizations

Zhen Song[1], Alain Karma[2], Hrayr S. Karagueuzian[1], James N. Weiss[1], Zhilin Qu[1].
[1]Department of Medicine (Cardiology), David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, [2]Physics, Northeastern University, Boston, MA, USA.

Early (EADs) and delayed (DADs) afterdepolarizations are triggers of cardiac arrhythmias. While DADs are caused by spontaneous Ca releases, EADs can originate from either voltage oscillations or Ca oscillations. In addition, in many situations, both EADs and DADs occur simultaneously. However, voltage and Ca are bi-directionally coupled, how this coupling affects EADs and the interactions between EADs and DADs are not well understood. Here we use an action potential (AP) model of ventricular myocytes with detailed spatiotemporal Ca cycling regulation to investigate the effects of voltage-Ca coupling on EAD and DAD genesis and their interactions. We increased the Ca window current or the late Na current to promote voltage oscillations and changed the RyR leakness and SERCA pump activity to promote Ca oscillations. By scanning the parameter space of voltage and Ca cycling, we found that: 1) EADs can be caused by either voltage oscillations or Ca oscillations. Ca oscillations also promote DADs and triggered activity; 2) An EAD can result from a secondary Ca release event due to transient SR Ca overload caused by very fast Ca re-uptake and/or a lengthened AP; when AP is short, this secondary release results in a DAD; 3) For certain parameters, partially decoupling voltage and Ca by removing the Ca-dependence of IKs suppresses both EADs and DADs, indicating that Ca and voltage interact synergistically to promote EADs and DADs; and 4) Ca oscillations are markedly reduced under voltage clamp conditions, further suggesting that Ca oscillations and voltage oscillations promote each other in a synergistic manner. In conclusion, the coupling of voltage and Ca promotes the formation of EADs and DADs and generates large amplitude EADs that are arrhythmogenic.

3189-Plat

Correlation between Ventricular Repolarisation Patterns and T-Wave Generation in Isolated Rabbit Hearts using Panoramic Imaging

Andrew Allan, Godfrey Smith, Francis Burton.
University of Glasgow, Glasgow, United Kingdom.

It is generally accepted that the normal T-wave is the manifestation of repolarisation gradients in the ventricles of the heart in an endocardial- epicardial direction. However, several studies contradict these findings, attributing T-wave polarity to apico-basal and/or interventricular repolarisation gradients. Consequently, the mechanisms responsible for the T-wave under both normal and pathological conditions remain unclear. In this study, isolated Langendorff-perfused rabbit hearts (37°C) were suspended in a customized chamber containing a frame that allowed the heart to be rotated to $\pm 120^{\circ}$ from a central position. Epicardial voltage was monitored by staining the heart with voltage sensitive dye (di-4-ANEPPS). Illumination at 480nm was provided by an array of LEDs (OptoLED, Cairn Research Ltd.). Emitted fluorescence was collected, filtered with 590nm long-pass filter and focused on a CCD chip (Redshirt Imaging, Decatur, GA) which acquired images (80x80 pixels) every 1ms. Recordings were made from 3 sides to obtain a panoramic map of cardiac electrical activity. Simultaneously, pseudo-ECG recordings (equivalent to Lead I) were recorded while pacing via electrodes placed on the right atrium. Epicardial ventricular repolarisation patterns were correlated to corresponding ECG recordings. In contrast to some previous studies, less than 5% of the epicardium (right and left ventricles) was repolarised by the peak of the T-wave. In addition, the presence of an apico-basal repolarisation gradient on the left ventricle (LV) developed in the latter half of the T-wave. An inverse relationship between APD and activation time was observed across the LV suggesting that activation pattern is a determinant of epicardial APD. In conclusion, the lack of a prominent epicardial repolarisation gradient during the first half of the T-wave suggests that regions other than the epicardial surface are responsible for the initial deflection.

3190-Plat

Enhanced Differentiation of Stem Cell Derived Cardiac Myocytes by Electronic Expression of IK1 Reveals an Atrial-Specific Kv1.5-Like Current

Aaron D. Kaplan[1], Agnieszka Lis[1], Thomas R. Cimato[2], Emmanuel S. Tzanakakis[3], Qinlian Zhou[1], Michael J. Morales[1], Randall L. Rasmusson[1], **Glenna C.L. Bett**[1].
[1]Physiology and Biophysics, SUNY, Buffalo, NY, USA, [2]Medicine, SUNY, Buffalo, NY, USA, [3]Chemical Engineering, SUNY, Buffalo, NY, USA.

Human induced pluripotent stem cell derived cardiac myocytes (h-iPSC-CMs) are a major advance in drug safety testing and research into human cardiac electrophysiology. However, these cells have potential limitations when used for quantitative action potential (AP) analysis. These cells are a mixture of atrial and ventricular cell types, which can be distinguished, to some extent, by action potential (AP) morphology. However, the spontaneous activity of h-iPSC-CMs results in parameter variability and anomalous pharmacological responses, leading to cell misidentification. The spontaneous behavior is largely due to the absence of an IK1 inwardly rectifying potassium channel (IK1).

We examined the effect of using an in silico interface to electronically express this missing IK1. An in silico interface was developed to express synthetic IK1 in cells under whole cell voltage clamp using a variant of the dynamic clamp approach. Electronic IK1 expression resulted in a stable physiological resting potential, eliminated spontaneous activity, reduced spontaneous early and delayed after depolarizations, and decreased AP variability. Stimulated APs had a rapid upstroke and spike and dome morphology, and the readily recognizable repolarization attributes of ventricular and atrial cells. When examining the late components of outward current, we found that APs classified on the basis of AP morphology as ''atrial-like'' had a significantly larger sustained outward Kv1.5-like component at +50 mV than ''ventricular-like'' APs (in pA/pF: 1.76 ± 0.47 (n=6) vs. 0.74 ± 0.07 (n=7), $p<0.05$) but similar peak outward currents: (4.00 ± 0.36 (n=6) vs. 3.71 ± 0.21 (n=7), $p>0.4$). These data indicate that h-IPSC-CM myocytes with synthetic expression of IK1 can easily be differentiated into ventricular-like and atrial-like myocytes by AP morphology, and that atrial-like cells exhibit an atrial-specific current.

3191-Plat

PLB Drives the Kinetics of the Ca^{2+} Clock in Mouse Isolated Sinoatrial Nodal Cells and the Intrinsic Heart Rate in vivo

Syevda Sirenko, Ismayil Ahmet, Edward G. Lakatta.
NIA, Baltimore, MD, USA.

The coupled-clock pacemaker cell theory predicts that changes in the characteristics of Ca^{2+} pumping into sarcoplasmic reticulum (SR) underlie changes in sinoatrial nodal cells (SANC) AP firing rate and the intrinsic heart rate (IHR) in vivo. We hypothesized that the absence of phospholamban (PLB) increases Ca^{2+} clock performance and the IHR. We measured: the IHR during double autonomic blockade in 3MO $PLN^{-/-}$ mice and in age-matched control (WT); the SR Ca^{2+} load (by caffeine spritz) and spontaneous ryanodine receptor (RyR) local Ca^{2+} releases (LCRs) by confocal microscopy in permeabilized single SANC clamped at 100 nM $[Ca^{2+}]_i$. The IHR of $PLN^{-/-}$ was significantly higher than WT (Fig. A) and was accompanied by an increase in SR Ca^{2+} loading (Fig. B). The LCR frequency, duration and Ca^{2+} signals amplitudes (μm*ms*ΔCa^{2+} nmol/L) were markedly increased (Fig. B); the integrated Ca^{2+} signal of the LCR Ca^{2+} ensemble (μm*ms*ΔCa^{2+} nmol/L) was 3 fold higher in $PLN^{-/-}$ vs. WT ($p<0.05$, n=7-10 cells). **Conclusion:** PLB regulation of SR Ca^{2+} cycling is a crucial determinant of Ca^{2+} clock function in vitro and of the IHR in vivo.

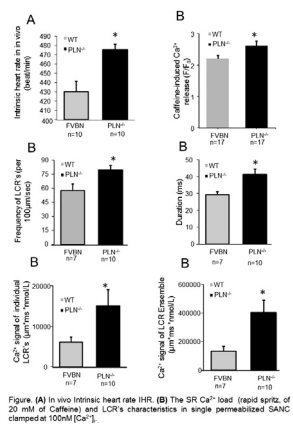

Figure. (A) In vivo intrinsic heart rate IHR. (B) The SR Ca^{2+} load (rapid spritz of 20 mM of Caffeine) and LCR's characteristics in single permeabilized SANC clamped at 100nM $[Ca^{2+}]_i$.

3192-Plat

Burst Pacemaker Activity in NCX1 Knockout Mice: Is it Funny Current?

Angelo Giovanni Torrente[1], Audrey Zaini[1], Ashley Rosenberg[1], Rui Zhang[1], Jeanney Kang[1], Kenneth D. Philipson[2], Joshua I. Goldhaber[1].
[1]Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

The cardiac Sodium-Calcium exchanger (NCX1) is the dominant Ca efflux protein in SinoAtrial Node (SAN) cells. NCX1 has also been implicated in the generation of pacemaker activity, through the ''Ca clock''. To study SAN pacing in the absence of NCX1, we created an atrial-specific NCX1 KO mouse using a Cre/loxP system under the control of the endogenous sarcolipin promoter. NCX1 KO mice have complete AV block and no P waves. The latter is caused by conduction interference between SAN and atria. To test the hypothesis that SAN pacemaking can be maintained by funny current (I_f) in the absence of NCX1, we recorded Ca transients (using Cal 520 and high speed 2D confocal imaging) in an ex vivo tissuepreparation that included the SAN, right atrium and left atrium. At ~36°C, the SAN pacemaker rate was 5.3 ± 0.3Hz in WT (n=14) but only 2 ± 0.2Hz (n=17) in KO. The pattern of pacing in KO was characterized by frequent bursts of Ca transients alternating with long pauses.